REMARKS

Claims 1-20 and 58 are pending and under examination.

The rejection of claims 1-20 and 58 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement is respectfully traversed. Applicants respectfully maintain, for the reasons of record, that the specification provides sufficient description and guidance to enable the claimed methods.

Claims 1 to 20 and 58 are directed to methods of differentiating progenitor cells including human progenitor cells such as human embryonic stem (ES) cells. Applicants maintain, for the reasons of record that, in view of the teachings in the specification, one skilled in the art would have been able to practice the claimed methods of differentiating progenitor cells without undue experimentation. As argued previously, the specification teaches obtaining a variety of mammalian embryonic stem cells including stem cells from mice, cows, primates and humans, for example, human stem cells from infant cord blood or from adult peripheral blood (page 47, line 5, to page 51, line 12). As further guidance to the skilled person, the specification teaches methods for introducing a nucleic acid molecule into human or other progenitor cells (page 54, line 4, to page 55, line 17). As taught in the specification, well-known methods for introducing a nucleic acid molecule into progenitor cells such as embryonic stem cells include microinjection, electroporation, lipofection and viral-mediated techniques such as lentiviral and retroviral transduction (page 54, line 4, to page 55, line 17). Thus, the specification provides guidance to the skilled person regarding how to make and use the invention, including how to practice the invention with human progenitor cells.

Applicants point to the evidence submitted as Exhibit A with the previous response filed September 3, 2004, Cheng et al., Blood 92: 83-92 (1998), as corroboration that one skilled in the art would have been able to introduce a nucleic acid molecule into human progenitor cells. This article, which was published prior to the June 2000 priority date of the subject application, reports an optimized retroviral gene-transfer protocol clinically applicable to gene transfer into human hematopoietic stem cells (page 83, abstract). In particular, the 1998 paper by Cheng et al. reports the improved efficiency of their retroviral-mediated gene transfer protocol; as indicated at page 86, second column, greater than 30% of the CD34+/Lin- stem cells expressed the NGFR transgene (see, also, page 87, Figure 3). These result confirm the teachings of the specification indicating that

retroviral vectors can be useful in the invention (see, for example, specification at page 55, lines 1-17). In sum, the 1998 publication by Cheng et al. provided as Exhibit A in the previous response substantiates that one skilled in the art would have been able to introduce a nucleic acid molecule into human progenitor cells such as human hematopoietic stem cells at the time the invention was made.

Furthermore, Applicants maintain that, as set forth in the specification, one skilled in the art would have been able to use other routine techniques such as electroporation to introduce a nucleic acid molecule into human or other progenitor cells. In this regard, the specification teaches that electroporation can be useful for introducing a nucleic acid molecule into embryonic stem cells in a method of the invention (page 54, lines 6-10) and that the invention can be practiced with any of a variety of embryonic stem cells including human embryonic stem cells (page 28, lines 7-22). Applicants maintain that, irregardless of whether the methods of the invention have been reduced to practice using electroporation, the specification enables the invention by teaching that electroporation and other routine transfection methods can be useful in introducing a nucleic acid molecule into progenitor cells in a method of the invention.

Subsequent results also corroborate that electroporation would have been useful for introducing a nucleic acid molecule into human progenitor cells in accordance with the teachings in the specification. Applicants direct the Examiner's attention to Exhibit B filed with the prevoius response, Eiges et al., Curr. Biol. 11:514-518 (2001), in which the efficiency of transfection into H9 human ES cells was assessed by determining luciferase reporter gene activity. As shown at page 515, Figure 1 of Eiges et al., luciferase reporter gene activity was observed in electroporated H9 human ES cells, indicating that these human progenitor cells were successfully transfected. Furthermore, Zwaka and Thomson, Nat. Biotechnol. 21:319-321 (2003), attached as Exhibit C in the previous response, demonstrates that electroporation can be used to produce stably transfected human ES cells. At page 2, first sentence, Zwaka and Thompson reports that electroporation of human ES cells yielded a stable transfection rate of 5.6×10^{-5}. See, also, page 1, second column, second full paragraph, of Zwaka and Thompson, which indicates that, following electroporation of 1.5×10^{7} human ES cells and selection with G418, 103 G418-resistant clones were obtained. Thus, subsequent results corroborate the teachings of the specification by demonstrating that only routine

methods such as electroporation would have been required for the skilled person to practice the claimed invention with human progenitor cells.

Applicants maintain, as taught in the specification, that the claimed methods of differentiating progenitor cells can be practiced using either permanent or transient expression (specification at page 55, lines 14-17). For example, as is well known in the art and exemplified in the specification, antibiotic selection can be used to prepare progenitor cells in which a large percentage or all of the cells are selected to contain the introduced MEF2-encoding nucleic acid molecule. In this regard, the specification exemplifies stable transfection of P19 embryonic stem cells with MEF2C produced using selection with the antibiotic Geneticin (page 68, line 19, to page 69, line 4). Again, the specification provides guidance to the skilled person regarding the use of electroporation as well as permanent expression of MEF2-encoding nucleic acid molecules. Subsequent work, as described above in Zwaka and Thompson, corroborates that electroporation can be used to prepare stably transfected human progenitor cells. Thus, as taught in the specification, stable as well as transient transfection can be useful in the claimed methods of differentiating progenitor cells.

Regarding the efficiency of transfection of human stem cells, Applicants maintain that the claimed methods of differentiating progenitor cells do not require a particularly "high efficiency" of transfection of human or other progenitor cells. In particular, introducing a MEF2-encoding nucleic acid molecule into a small number of progenitor cells can be followed by antibiotic selection for the presence of the MEF2-encoding nucleic acid molecule. Thus, in combination with selection, low efficiency transfection of progenitor cells can be useful in the invention. Again, this point of view is corroborated by results of Zwaka and Thompson discussed above in which a transfection rate of about 5×10^{-5} was sufficient to produce more than 100 antibiotic-resistant colonies of human progenitor cells containing the transfected nucleic acid molecule. These results corroborate that, even if progenitor cells were not transfected with particularly high efficiency, one skilled in the art would have been able produce a population of progenitor cells predominantly or uniformly containing a MEF2-encoding nucleic acid molecule using routine methods.

Applicants maintain that it is well known in the art that growth and differentiation factors are present *in vivo* in the diseased or injured tissue environment. A number of factors with

neurotrophic activies are known in the art including nerve growth factor (NGF), brain-derived growth factor (BDNF), ciliary neurotrophic factor (CNTF) and others (see, for example, Jacowski, Brit. J. Neurosurg. 9:303-317 (1995), attached as Exhibit D in the previous response, paragraph spanning pages 308 and 309). Furthermore, the expression of growth and differentiation factors can be recapitulated or upregulated as compared to the expression of the factors in the normal adult nervous system. As an example, Jacowski report that peripheral nerve transection results in large quantities of NGR being produced by supporting Schwann cells and that central nervous system (CNS) injury "causes a time-dependent increase in neuronotrophic activity at a lesion site" (page 309, first column, second full paragraph). Thus, in contrast to the assertion in the Office Action, increased levels of nerve growth and differentiation factors are expected in diseased or injured tissue as compared to healthy tissue.

As additional evidence that appropriate environmental cures can be present in diseased or injured tissue, Applicants respectfully point out that the results of McDonald et al., "Transplanted Embryonic Stem Cells Survive, Differentiate and Promote Recovery in Injured Rat Spinal Cord," Nature Med. 5:1410-1412 (1999), submitted with the previous response as Exhibit E, were achieved in mice subject to traumatic injury, specifically, impact injury of the spinal cord using a weight-drop device (page 1412, first column, second full paragraph). The results of McDonald et al. demonstrate that mouse embryonic stem cells survived and differentiated into astrocytes, oligodendrocytes and neurons following transplantation were achieved in acutely injured rather than healthy animals (page 1410, abstract, and second column).

Applicants' position also is supported by Liu et al., "Embryonic Stem Cells Differentiate into Oligodendrocytes and Myelinate in Culture and after Spinal Cord Transplantation," Proc. Natl. Acad. Sci. USA 97:6126-6131 (2000), submitted with the previous response as Exhibit F. In particular, Liu et al. report embryonic stem cell transplantation experiments in two animal models: an "injury model" based on chemical demyelination and a "noninjury model" based on mice genetically deficient in myelin basic protein, emphasizing that the purpose of their studies was to determine whether embryonic stem cells can survive transplantation into both injured and uninjured adult spinal cord (page 6126, second column, first complete paragraph; emphasis added). Liu et al. conclude that their studies demonstrate the ability of embryonic stem cell-derived oligodendrocytes

to "replace lost myelin in the injured adult CNS (page 6126, abstract, penultimate sentence; emphasis added). Similarly, Liu et al. conclude that

> Our study demonstrates the ability of ES cell-derived oligodendrocytes to myelinate *in vitro* and to show that ES cells survive and myelinate axons in the mature and injured CNS after transplantation.... In particular, we demonstrate that injured, demyelinated areas of the adult CNS may preferentially stimulate oligodendrocyte differentiation/survival (page 6131, first paragraph).

Applicants further submit that the specification provides sufficient description and guidance for the claimed methods and that one skilled in the art would have been able to practice the invention as claimed without undue experimentation. Applicants respectfully disagree that the claimed methods are unpredictable in light of Rossi and Cattaneo, Nat. Rev. Neurosci. 3:401-409 (2002), Cao et al., J. Neurosci. Res. 68:501-510 (2002), Milward et al., J. Neurosci. Res. 50:862-871 (1999), and/or Mehler et al., Arch. Neurol. 56:780-784 (1999).

Applicants respectfully maintain that the specification provides sufficient description and guidance to enable the claimed methods. Accordingly, Applicants respectfully request that this rejection be withdrawn.

In light of the amendments and remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. The Examiner is invited to call the undersigned agent if there are any questions.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

MCDERMOTT WILL & EMERY LLP

Deborah L. Cadena
Registration No. 44,048

4370 La Jolla Village Drive, Suite 700
San Diego, CA 92122
Phone: 858.535.9001 DLC:llf
Facsimile: 858.597.1585
Date: May 31, 2005

Please recognize our Customer No. 41552 as our correspondence address.